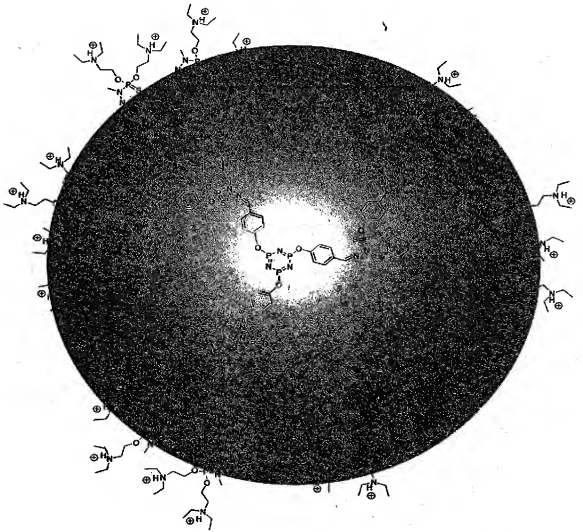
B Christensen and P M H Heegaard

Dendrimers in Medicine and Biotechnology

New Molecular Tools



RSC Publishing

The front cover image is based on a design from the inside front cover of *Chem Commun.*, 2005, 289–416, from a paper by J. Wu, J. Zhou, F. Qu, P. Bao, Y. Zhang and I. Peng, pg 313.

ISBN-10: 0-85404-852-9
ISBN-13: 978-0-85404-852-6

A catalogue record for this book is available from the British Library

Published by The Royal Society of Chemistry,
Thomas Graham House, Science Park, Milton Road,
Cambridge CB4 0WF, UK

Registered Charity Number 207890

For further information see our web site at www.rsc.org

Typeset by Macmillan India Ltd, Bangalore, India
Printed by Henry Ling Ltd, Dorchester, Dorset, UK

Table of Contents

CHAPTER 1

Dendrimers: Design, Synthesis and Chemical Properties

1.1 Introduction

The dendritic structure is a widespread motif in nature often utilised where a particular function needs to be exposed or enhanced. Above ground, trees use dendritic motifs to enhance the exposure of their leaves to the sunlight, which is crucial to maintain life and growth via the photosynthesis. The shade of the tree crown creates a microenvironment maintaining higher humidity and more stable temperatures throughout the day compared to the surroundings. Also beneath ground, the trees have a maximum need to expose a large functional surface when collecting water from the soil. A large dendritic network of roots provides an excellent motif for that purpose (Figure 1.1).

In the "design" of animals and humans, evolution often ends up creating dendritic solutions to enhance particular properties. When breathing air into our lungs the air

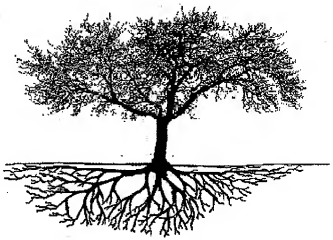


Figure 1.1

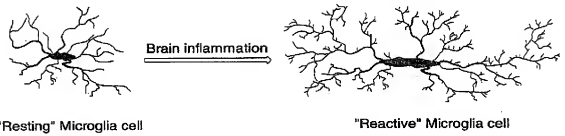


Figure 1.2 *Activation of a Microglia cell during a pathological state in the brain*

passes through a tremendous dendritic network of bronchioles and alveoli in order to give maximum surface for the transfer of oxygen into the bloodstream. Also the arterial network transporting the oxidised blood to the different organs progress into dendritic patterns, before the blood is transported back to the heart via the venous system.[1] The central nervous system and the brain consist of a large amount of cells growing into dendritic structures in order to gain the largest exchange of material (and information) with the surrounding tissue. Microglia cells serving as multifunctional helper cells in the brain, form dendritic strucures when activated during pathological or degenerative states in the brain (Figure 1.2). Also here the dendritic structure ensures maximum delivery of secreted anti-inflammatory interleukins to the diseased brain tissue.

Another striking example of dendritic structures in nature discovered just recently, is the tremendous number of foot-hairs on the Gecko's feet. These foot-hairs "setae" split up into an impressive dendritic network of tiny foot hairs "spatulae", enabling the Gecko to "stick" to surfaces through dry adhesion without the need of humidity to create surface tension. Examinations of the Gecko's foot-hairs have revealed that the structures of the millions of end foot-hairs are so microscopic that the adhesion between the surface and the gecko foot is thought to be achieved by weak attractive quantum chemical forces from molecules in each foot-hair interacting with molecules of the surface, the so-called Van der Waal forces.[2] By applying a dendritic pattern, the enhancement of a certain function can sometimes greatly exceed the sum of single entities carried on the surface, because of the synergy gained by a dendritic presentation of a function. So nature has, indeed, applied dendritic structures throughout evolution with great success.

In synthetic organic chemistry the creation and design of dendritic compounds is a relatively new field. The first successful attempt to create and design dendritic structures by organic synthesis was carried out by Vögtle and co-workers[3] in 1978. These relatively small molecules were initially named "cascade molecules" and already then Vögtle and co-workers saw the perspectives in using these polymers as, *e.g.* molecular containers for smaller molecules. However, after this first report, several years passed before the field was taken up by Tomalia's group at Dow Chemicals. They had during the years developed a new class of amide containing cascade polymers, which brought these hitherto quite small molecular motifs into well-defined macromolecular dendritic structures. Tomalia and co-workers[4,5] baptised this new class of macromolecules "dendrimers" built up from two Greek words "dendros" meaning "tree" or "branch" and "meros" meaning "part" in Greek. Later

refinement and development of synthetic tools enabled the scientists also to synthesise macromolecular structures relying on the original "Vögtle cascade motif".[6,7]

Parallel to polymer chemists taking this new class of compounds into use, dendritic structures also started to emerge in the "biosphere", where J. P. Tam in 1988 developed intriguing dendritic structures based on branched natural amino acid monomers thereby creating macromolecular dendritic peptide structures commonly referred to as "Multiple Antigen Peptide". The Multiple Antigen Peptide is, as we shall see later, a special type of dendrimer.[8]

Dendrimers are also sometimes denoted as "arboroles", "arborescent polymers" or more broadly "hyperbranched polymers", although dendrimers having a well-defined finite molecular structure, should be considered a sub-group of hyperbranched polymers. After the initial reports the papers published on the synthesis, design and uses of dendrimers in chemistry as well as in biological field has had an exponential increase in numbers.[9–14]

1.2 Terms and Nomenclature in Dendrimer Chemistry

Dendrimer chemistry, as other specialised research fields, has its own terms and abbreviations. Furthermore, a more brief structural nomenclature is applied to describe the different chemical events taking place at the dendrimer surface. In the following section a number of terms and abbreviations common in dendrimer chemistry will be explained, and a brief structural nomenclature will be introduced.

Hyperbranched polymers is a term describing a major class of polymers mostly achieved by incoherent polymerisation of AB_n ($n \geq 2$) monomers, often utilising one-pot reactions. Dendrimers having a well-defined finite structure belongs to a special case of hyperbranched polymers (see Figure 1.3). To enhance the availability of dendritic structures, hyperbranched polymers are for some purposes used as dendrimer "mimics", because of their more facile synthesis. However, being polydisperse, these types

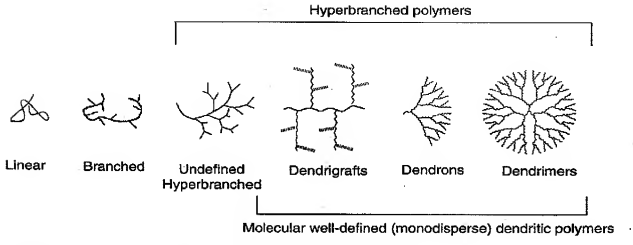


Figure 1.3 *Evolution of polymers towards dendritic structures*

of polymers are not suitable to study chemical phenomena, which generally require a well-defined chemical motif enabling the scientist to analyse the chemical events taking place. The physicochemical properties of the undefined hyperbranched polymers are intermediate between dendrimers and linear polymers.[15]

Dendrigrafts are class of dendritic polymers like dendrimers that can be constructed with a well-defined molecular structure, *i.e.* being monodisperse. However, in contrast to dendrimers, dendrigrafts are centred around a linear polymer chain, to which branches consisting of copolymer chains are attached. These copolymer chains are further modified with other copolymer chains and so on, giving a hyperbranched motif built up by a finite number of combined polymers.[16] Whereas the dendrimer resembles a tree in structure, the core part of a dendrigraft to some extent resembles the structure of a palm-tree.

Dendrons is the term used about a dendritic wedge without a core, the dendrimer can be prepared from assembling two or more dendrons. As we shall see later, dendrons are very useful tools in the synthesis of dendrimers by the segment coupling strategy (convergent synthesis). A class of dendrons, which is commercially available and has been applied with great success in the covalent and non-covalent assembly of dendrimers, are the "Fréchet-type dendrons".[17–19] These are dendritic wedges built up by hyperbranched polybenzylether structure, like the Fréchet-type dendrimers.[17–19] These dendrons have been used in the creation of numerous of dendrimers having different structures and functions.

Generation is common for all dendrimer designs and the hyperbranching when going from the centre of the dendrimer towards the periphery, resulting in homo-structural layers between the focal points (branching points). The number of focal points when going from the core towards the dendrimer surface is the generation number (Figure 1.4). That is a dendrimer having five focal points when going from the centre to the periphery is denoted as the 5th generation dendrimer. Here, we abbreviate this term to simply a G5-dendrimer, *e.g.* a 5th generation polypropylene imine and a polyamidoamine dendrimer is abbreviated to a "G5-PPI-" and "G5-PAMAM" dendrimer, respectively. The core part of the dendrimer is sometimes denoted generation "zero", or in the terminology presented here "G0". The core structure thus presents no focal points, as hydrogen substituents are not considered focal points. Thus, in PPI dendrimers, 1,4-diaminobutane represents the G0 core-structure and in PAMAM Starburst dendrimers ammonia represents the G0 core-structure. Intermediates during the dendrimer synthesis are sometimes denoted half-generations, a well-known example is the carboxylic acid-terminated PAMAM dendrimers which, as we shall see later, sometimes have properties preferable to the amino-terminated dendrimers when applied to biological systems.

Shell: The dendrimer shell is the homo-structural spatial segment between the focal points, the "generation space". The "outer shell" is the space between the last outer branching point and the surface. The "inner shells" are generally referred to as the dendrimer interior.

Pincer: In dendrimers, the outer shell consists of a varying number of pincers created by the last focal point before reaching the dendrimer surface. In PPI and PAMAM dendrimers the number of pincers is half the number of surface groups (because in these dendrimers the chain divides into two chains in each focal point).

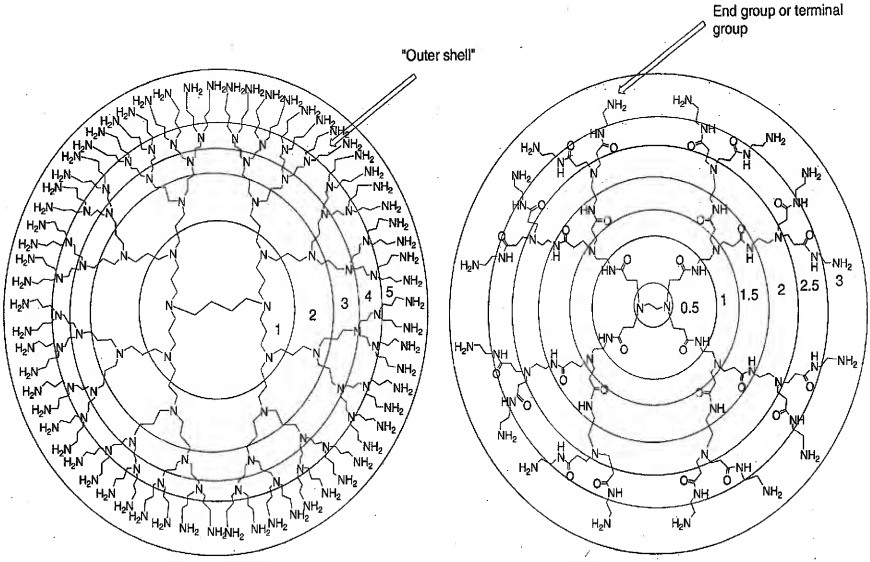


Figure 1.4 *PPI of PAMAM dendrimers with generation of shell depiction*

End-group is also generally referred to as the "terminal group" or the "surface group" of the dendrimer. The word surface group is slightly more inaccurate, in the sense that the dendrimer branches can sometimes fold into the interior of the dendrimer. Dendrimers having amine end-groups are termed "amino-terminated dendrimers".

MAP-dendrimers stand for "Multiple Antigen Peptide", and is a dendron-like molecular construct based upon a polylysine skeleton. Lysine with its alkylamino side-chain serves as a good monomer for the introduction of numerous of branching points. This type of dendrimer was introduced by J. P. Tam in 1988,[8] has predominantly found its use in biological applications, *e.g.* vaccine and diagnostic research. MAP was in its original design a "tree shaped" dendron without a core. However, whole dendrimers have been synthesised based upon this motif either by segmental coupling in solution using dendrons or stepwise by solid-phase synthesis.[20]

PPI-dendrimers stand for "Poly (Propylene Imine)" describing the propyl amine spacer moieties in the oldest known dendrimer type developed initially by Vögtle.[3] These dendrimers are generally poly-alkylamines having primary amines as end-groups, the dendrimer interior consists of numerous of tertiary tris-propylene amines. PPI dendrimers are commercially available up to G5, and has found widespread applications in material science as well as in biology. As an alternative name to PPI, POPAM is sometimes used to describe this class of dendrimers. POPAM stands for POly (Propylene AMine) which closely resembles the PPI abbreviation. In addition, these dendrimers are also sometimes denoted "DAB-dendrimers" where DAB refers to the core structure which is usually based on DiAminoButane.

PEI-dendrimers is a less common sub-class of PPI dendrimers based on Poly (Ethylene Imine) dendritic branches. The core structure in these dendrimers are diamino ethane or diamino propane.

PAMAM-dendrimers stand for PolyAMido-AMine, and refers to one of the original dendrimer types built up by polyamide branches with tertiary amines as focal points. After the initial report by Tomalia and co-workers[4,5] in the mid-1980s PAMAM dendrimers have, as the PPI dendrimers, found wide use in science. PAMAM dendrimers are commercially available, usually as methanol solutions. The PAMAM dendrimers can be obtained having terminal or surface amino groups (full generations) or carboxylic acid groups (half-generations). PAMAM dendrimers are commercially available up to generation 10.[17]

Starburst dendrimers is applied as a trademark name for a sub-class of PAMAM dendrimers based on a tris-aminoethylene-imine core. The name refers to the star-like pattern observed when looking at the structure of the high-generation dendrimers of this type in two-dimensions. These dendrimers are usually known under the abbreviation PAMAM (Starburst) or just Starburst.

Fréchet-type dendrimers is a more recent type of dendrimer developed by Hawker and Fréchet[17–19] based on a poly-benzylether hyperbranched skeleton. This type of dendrimer can be symmetric or built up asymmetrically consisting of 2 or 3 parts of segmental elements (dendrons) with, *e.g.* different generation or surface motif. These dendrimers usually have carboxylic acid groups as surface groups, serving as a good anchoring point for further surface functionalisation, and as polar surface groups to increase the solubility of this hydrophobic dendrimer type in polar solvents or aqueous media.

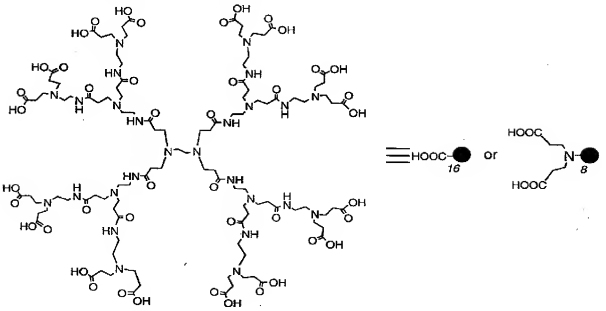


Figure 1.5 *"Black ball" symbol for a 2.5 G-PAMAM dendrimer*

"Black ball" nomenclature: Because of the large molecular structure of a dendrimer, the full picture of, *e.g.* reactions taking place on the dendrimer surface or in the outer shell can be difficult to depict. A way to facilitate the depiction of these macromolecules is by showing the inner (and unmodified) part of the dendrimer as a "black ball". Depending on whether the reaction takes place at the surface groups or in the outer shell, the appropriate part of the molecular motif, *e.g.* the outer pincers, may be fully drawn out to give a concise picture of a reaction involving the outer shell (see Figure 1.5). In this way the picture of reactions taking place at the dendrimer surface or in the outer shell is greatly simplified.

1.3 Dendrimer Design

After the initial reports and development of these unique well-defined structures, chemists have begun to develop an excessive number of different designs of dendrimers for a wide variety of applications. Newkome and co-workers[22] developed the unimolecular micelle consisting of an almost pure hydrocarbon scaffold, Majoral and Caminade introduced the multivalent phosphorus to create intriguing new dendrimeric designs and dendrimers having new properties. Other third period elements like silicon and sulfur have been implemented in the dendritic structures resulting in dendrimers having properties quite different from the classical PAMAM and PPI designs.[23] The monomers applied in the build-up of a dendrimer are generally based on pure synthetic monomers having alkyl or aromatic moieties, but biological relevant molecules like carbohydrates,[24] amino acids[20] and nucleotides[25–27] have been applied as monomers as well (Figure 1.6).

Using biological relevant monomers as building blocks presents an intriguing opportunity to incorporate biological recognition properties into the dendrimer.[20,24]

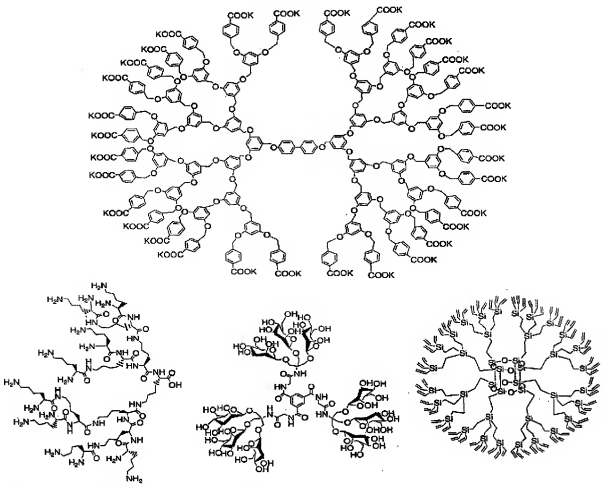


Figure 1.6 *Different dendrimer designs. Top: G3-Fréchet-type dendrimer. Bottom from the right: MAP dendron, glycodendrimer and a silicon-based dendrimer*

As we shall see, also metal ions serve as good focal points and have found extensive use in various functional dendrimer designs as well as in the synthesis of dendrimers by self-assembly.[28]

1.4 Dendrimer Synthesis

Divergent dendrimer synthesis: In the early years of dendrimers, the synthetic approach to synthesise the two major dendrimer designs, the PPI and PAMAM, relied on a stepwise "divergent" strategy. In the divergent approach, the construction of the dendrimer takes place in a stepwise manner starting from the core and building up the molecule towards the periphery using two basic operations (1) coupling of the monomer and (2) deprotection or transformation of the monomer end-group to create a new reactive surface functionality and then coupling of a new monomer *etc.*, in a manner, somewhat similar to that known from solid-phase synthesis of peptides or oligonucleotides.

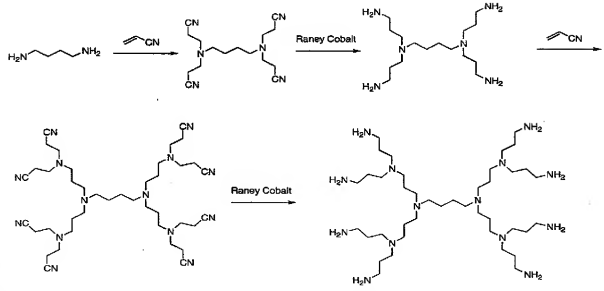


Figure 1.7 *Poly(propylene imine) dendrimer synthesis by divergent strategy*

For the poly (propyleneimine) dendrimers, which are based on a skeleton of poly alkylamines, where each nitrogen atom serves as a branching point, the synthetic basic operations consist of repeated double alkylation of the amines with acrylonitrile by "Michael addition" results in a branched alkyl chain structure. Subsequent reduction yields a new set of primary amines, which may then be double alkylated to provide further branching *etc.* (Figure 1.7)[7]

PAMAM dendrimers being based on a dendritic mixed structure of tertiary alkylamines as branching points and secondary amides as chain extension points was synthesised by Michael alkylation of the amine with acrylic acid methyl ester to yield a tertiary amine as the branching point followed by aminolysis of the resulting methyl ester by ethylene diamine.

The divergent synthesis was initially applied extensively in the synthesis of PPI and PAMAM dendrimers, but has also found wide use in the synthesis of dendrimers having other structural designs, *e.g.* dendrimers containing third period heteroatoms such as silicium and phosphorous.[23] Divergent synthesis of dendrimers consisting of nucleotide building blocks has been reported by Hudson and co-workers.[25] The divergent stepwise approach in the synthesis of nucleotide dendrimers and dendrons is interesting from a biochemical perspective as it may mimic the synthesis of naturally occuring *lariat* and *forked introns* in microbiology.[25]

To discriminate between the divergent build-up of a linear molecule, *e.g.* a peptide/protein in a stepwise manner, and the proliferating build-up of a dendrimer also by a divergent methodology, Tomalia and co-workers have applied the term "Amplified Geneologically Directed Synthesis" or A-GDS to describe divergent dendritic synthesis, as an opposite to a "Linear Geneologically Directed Synthesis" (L-GDS) performed in, *e.g.* Merrifield solid-phase peptide synthesis (Figure 1.8).[29]

There are two major problems when dealing with divergent synthesis of dendrimers, (1) the number of reaction points increases rapidly throughout the synthesis

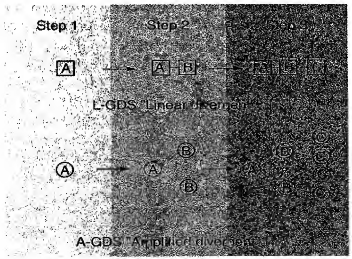


Figure 1.8

of the dendrimer, starting with 2 points in a G0-PPI dendrimer and ending up with 64 for G5-PPI dendrimer. This rapid increase in number of end-groups to be functionalised, combined with the following rapid increase in molecular weight resulting in slower reaction kinetics, makes the synthesis of the dendritic network to create higher generation dendrimers increasingly difficult, even when using high yielding reactions. Therefore the divergent approach may lead to increasing deletions throughout the growth of the dendrimer, resulting in numerous of defects in the higher generation dendrimer product. The synthesis of PPI dendrimers has to some extent been hampered by the creation of defects throughout the synthesis of higher generation dendrimers where it has been shown that the content of molecular perfect G5-PPI dendrimer in the product is only approximately 30%.[10] In the case of PPI dendrimers, the divergent approach is applied with most success in the synthesis of lower generation dendrimers (that is dendrimers upto G3). In case of the PPI dendrimers defects may also emerge in the final high generation product after synthesis, as a result of the PPI-structure being based on short spacer monomers. This creates an increasing molecularly crowded structure throughout the generations, leading to the loss of dendrimer branches and wedges because of increased susceptibility to, *e.g.* β-elimination reactions. Secondly, when performing divergent synthesis it is hard to separate the desired product from reactants or "deletion products", because of the great molecular similarity between these by-products and the desired product. Despite these drawbacks being observed predominantly in the synthesis of high generation PPI dendrimers, the divergent approach has been applied in the synthesis a large variety of different dendrimer designs with great success.

Generally the divergent approach leads to the synthesis of highly symmetric dendrimer molecules, however, recently scientists have taken up the possibility to create heterogeneously functionalised dendrimers by the divergent approach, leading to dendrimers having several types of functionalities bound to the surface.[30,31] This field is an exiting opportunity to use conventional dendrimers as scaffolds for different molecular functions.

Divergent dendrimer synthesis by self-assembly: Using biological building blocks gives a high degree of recognition, which can be used for highly specific self-assembly of the building blocks. Nilsen and co-workers[27] have used oligonucleotide building blocks in divergent self-assembly of dendrimers, followed by cross-binding to stabilise the self-assembled dendrimer construct. Although constituting an interesting example of a divergent segment-based synthesis, this method is quite complex due to the complex structure of the each building block. The building blocks consist of two annealed oligonucleotides annealing together at the mid-section, thus dividing out into four arms, which then each can be modified with monomers having complementary motifs on their arms and so on (Figure 1.9). The surface monomers may be modified with, *e.g.* a labelling group also by oligonucleotide annealing. Although this synthetic method does not result in perfect dendrimers, it still provides an intriguing alternative to divergent dendrimer synthesis relying on more traditional low-molecular monomers. This dendrimer design has been applied as scaffolds for biomolecules in diagnostics (see Chapter 5).

Convergent dendrimer synthesis: Segment coupling strategies began to be applied in peptide synthesis to circumvent the increasingly low reactivity experienced in stepwise divergent synthesis of large oligopeptides on solid-phase. With this new approach, peptide synthesis was taken a step further towards pure chemical synthesis of high molecular weight polypeptides and proteins (for a survey on convergent peptide/protein systhesis, see Ref. 32). This segmental coupling or convergent strategy also found its way into the creation of dendritic macromolecules, first implemented by Hawker and Fréchet[17–19] in their synthesis of poly-benzylether containing dendrimers which gave highly monodisperse dendrimer structures. A powerful alternative to the divergent

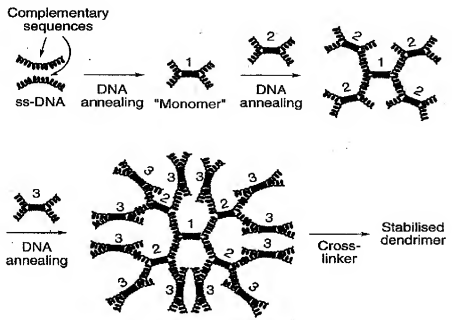


Figure 1.9 *Schematic depiction of divergent dendrimer synthesis based on oligonucleotode monomers using a self-assembly by oligonucleotide annealing, and after the final product is assembled, consolidation of the self-assembly by crosslinking*

approach had been introduced and this new tool was promptly taken up among other synthetic chemists working in the dendrimer field.

In contrast to the divergent method, the convergent method construct a dendrimer so to speak from the surface and inwards towards the core, by mostly "one to one" coupling of monomers thereby creating dendritic segments, dendrons, of increasing size as the synthesis progress. In this way the number of reactive sites during the proliferation process remains minimal leading to faster reaction rates and yields. Another advantage of this methodology is the large "molecular difference" between the reactant molecule and the product, facilitating the separation of the reactants from the product during the purification process. The final part of the convergent synthesis ends up at the core, where two or more dendritic segments (dendrons) are joined together, creating the dendrimer, the convergent strategy thus generally has an inverse propagation compared to the divergent strategy (Figure 1.10).

In addition, the convergent strategy is an obvious tool in the synthesis of asymmetric dendrimers, or dendrimers having mixed structural elements, where instead of coupling two equal segments in the final segment coupling reaction(s), different segments are coupled together to create dendrimers with heterogeneous morphologies.[33] This relatively easy approach to create heterogeneous dendrimers opens up to intriguing fields of incorporating several "active sites" in one dendrimer to create multifunctional macromolecular structures.

After its advent, the convergent strategy has also been used for the synthesis of a great variety of dendrimers having different core functionalities, where the core is introduced in the final step and modified with dendrons to create the complete dendrimer. This methodology facilitates the synthesis of dendrimers with different core functions, *e.g.* for fluorescence labelling or for the creation of artificial enzymes.

Convergent dendrimer synthesis by self-assembly: Much effort has been given to build up dendrimers in a non-covalent manner by convergent self-assembly of dendrons. The dendrons may contain functionalities capable of hydrogen bonding or metal complex bonding *etc.*, creating well-defined complexes having dendrimeric structures. The area was initially explored by Zimmerman's group[34] who built up dendrimers through self-assembly of dendrons capable of hydrogen bonding.

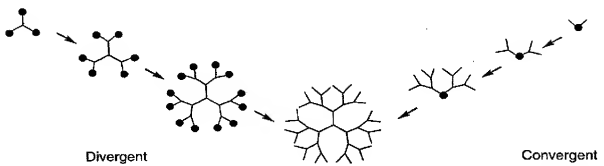


Figure 1.10 *Divergent versus convergent strategy, The black dots mark the "functionalising sites"*

When utilising hydrogen bonding as the "glue" to bind the dendrimer together, the general requirement is that the hydrogen bonding units chosen form complexes which are stable enough to be isolated. When designing these self-assembled products for biological applications as, *e.g.* drug delivery, or self-assembled drugs the hydrogen bonding keeping the segments together should be stable under highly polar physiological conditions, *i.e.* buffered aqueous media containing a high concentration of ions.

In the earliest attempts of non-covalent synthesis of dendrimers, Zimmerman's group[34] applied Fréchet dendrons containing bis-isophtalic acid, which in chloroform spontaneously formed hexameric aggregates through carboxylic acid–carboxylic acid hydrogen bonding. These hexameric aggregates were stable in apolar solvent like chloroform, but dissociated in more polar solvents like tetrahydrofuran and dimethyl sulfoxid, in which NMR only showed the existence of the corresponding monomers.

Another early report on the synthesis of dendrimers utilising self-assembly of hydrogen bonding dendrons was launched by Fréchet's group[35] who applied dendrons with complementary melamine and cyanuric acid functionalities for hydrogen bonding. These dendrons formed hexameric aggregates in apolar solvents, but as the "Zimmerman dendrons" these assemblies dissociated upon exposure to polar solvents. In order to increase the stability of self-assembled dendrimers in polar solvents, Zimmerman and his group[36] utilised the ureidodeazapterin moieties capable of forming exceptional strong hydrogen bonds, Frechet-type dendrons bound to this group via a spacer hydrogen bonded together and gave dimeric up to hexameric aggregates which had high stability both in apolar solvents like chloroform and in water.

An alternative to the development and design of synthetic molecular motifs capable of molecular recognition is the use of nature's own molecular motifs for highly specific molecular recognition. Single strand DNA (ss-DNA) forming stable complexes upon annealing with a complementary single DNA strands to form a DNA duplex has been applied as recognition motifs and bound to dendritic wedges. In this way two "complementary" dendrons each carrying one DNA-strand could be coupled together with high specificity forming a bi-dendronic dendrimer.[37]

A similar idea has been applied in the synthesis of supramolecular drugs for tumour targeting based on a "bi-dendrimer" by duplex formation of two differently functionalised dendrimers each containing a complementary oligonucleotide sequence (Figure 1.11)[38]

Metal ions with their Lewis acid properties may serve as good acceptors for appropriate electron pair donors attached to the dendrons, thus using the metal ion as the core for assembly of dendronic ligands. Dendrimers assembled around a lanthanide metal (*e.g.* Europium) have been created by Kawa and Fréchet.[39] The metal being in the core of the dendrimer experiences a microenvironment kept away from interacting with the surroundings, resulting in enhanced photoluminiscense. The site isolation retards energy transfer processes with the surroundings as well as the formation of metal clusters, which leads to the quenching observed in small ligand complexes (*e.g.* triacetates) of these elements.[39] Narayanan and

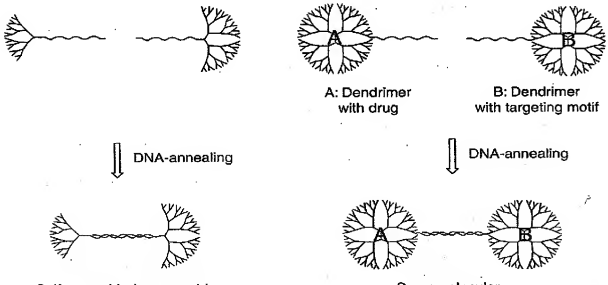


Figure 1.11 *Left: Schematic depiction of convergent dendron self-assembly creating asymmetric dendrimer constructs. Right: The use of ss-DNA in highly specific construction of multifunctional dendritic drugs*

Wiener[40] assembled dendrons around a Co^{3+} ion by formation of an octahedral complex where the metal was surrounded by three bidentate dendrons spreading out into six dendrimer branches. Cobalt is an extremely interesting element because of the large difference in properties when going from Co^{2+} generally forming quite unstable complexes with mostly tetrahedral symmetry compared to Co^{3+} which forms stable octahedral complexes. The use of transition metals as templates for dendrimer assembly presents the possibility to oxidise or reduce the metal centre, which may result in a new conformation or altered stability of the assembly, thereby creating a material responsive to oxidation or reduction from the surroundings.

An exciting aspect when applying weak binding forces compared to traditional covalent assembly, is the observation that even small molecular changes (or defects) in the respective monomers may have a strong effect on the ability for the final non-covalent dendrimer product to form.[41] In that sense this methodology closely resembles "natures way" of building up macromolecular structures, where even small "mutations" in, *e.g.* the amino acid side-chain motifs may lead to catastrophic consequences on the three-dimensional shape of the final protein and disable a particular biological function of that protein. The field of creating macroscopic dendrimeric nano-objects by self-assembly is a very important research area in order to get a closer understanding of the factors governing self-assembly processes, *e.g.* the molecular information concerning the shape of the final supramolecular product carried by the respective monomers. Furthermore, a deeper knowledge opens up to create molecular structures, which can change morphology and function upon

different stimuli. The non-covalent methodology is a very important approach for the creation of, *e.g.* functional biomaterials, capable of responding to the complex processes found in biological systems.

Self-assembly has been combined with conventional covalent synthesis of dendrimers by Shinkai and co-workers,[42] who used self-assembly of the dendrimers as templates for subsequent consolidation of the product by cross-linking the dendrimer together. In organic chemistry, the self-assembly processes leading to the right supramolecular product is at present time a relatively straightforward process due to the relatively simple supramolecular patterns and highly ordered structures of the building blocks. In nature, however, the self-assembly/consolidation process is a highly complicated matter, *e.g.* in the refolding of proteins from a denaturated state. Going from a highly disordered denaturated state to a highly ordered native state is a highly unfavourable process with respect to entropy (Figure 1.12).

In order to fold or refold the protein sequences into three-dimensional protein structures the unfolded or partially unfolded protein is taken up by a class of proteins called "Chaperones". Chaperones are cytoplasmic proteins that serve as templates in the folding process to give the final, and biological functional protein, and in preventing aggregate formation due to intermolecular hydrophobic interactions. The chaperones are also denoted "Heat Shock Proteins" because of their ability to prevent denaturation of proteins, which otherwise would be lethal, when our organisms are subjected to fever during illness.[43]

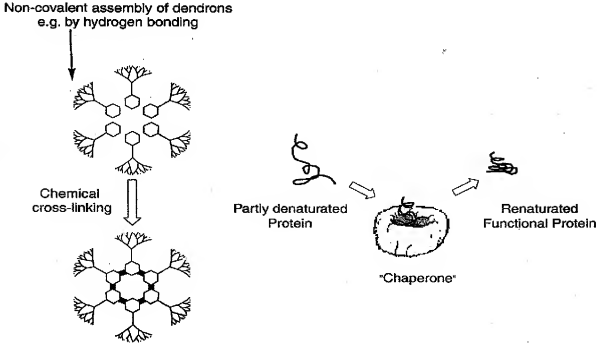


Figure 1.12 *Schematic depiction of, left: chemical assembly of a dendrimer via a self-assembly/in situ cross-binding strategy in comparison to the complex folding process of proteins in nature being mediated by Chaperones (right)*

1.5 Physicochemical Properties of Dendrimers

As the dendrimer grows, the different compartments of the dendritic structure begin to show distinct features which are amplified with increasing generation. The dendrimer structure may be divided into three parts:

- A multivalent surface, with a high number of functionalities. Dependent on the dendrimer generation, the surface may act as a borderline shielding off the dendrimer interior from the surroundings. This increasingly "closed" surface structure may result in reduced diffusion of solvent molecules into the dendrimer interior.
- The outer shell, which have a well-defined microenvironment, to some extent shielded from the surroundings by the dendrimer surface. The very high number of functionalities located on the surface and the outer shell are well-suited for host–guest interactions and catalysis where the close proximity of the functional motifs is important.
- The core, which as the dendrimer generation increases, gets increasingly shielded off from the surroundings by the dendritic wedges. The interior of the dendrimer creates a microenvironment which may have very different properties compared to the surroundings. For example as decribed elsewhere, water-soluble dendrimers with an apolar interior have been constructed to carry hydrophobic drugs in the bloodstream.[44]

The three parts of the dendrimer can specifically be tailored towards a desired molecular property or function of the dendrimer such as drug delivery, molecular sensors, enzyme mimics, *etc*.

When looking at the molecular size and properties of dendrimers, one soon observes that the molecular dimension of a higher generations dendrimer is comparable to medium-sized proteins (Table 1.1).[14]

Therefore, it was already early in the history of dendrimers suggested that these nanoscale polymers would serve as synthetic mimics of proteins.[45] However, the hyperbranched structure of the dendrimer creates a highly multivalent surface, exposing a much higher number of functional groups on the surface compared to proteins of similar molecular size (Table 1.1).

Also, the molecular weight of, *e.g.* a G6-PAMAM dendrimer is only around half of that of a protein of comparable molecular size (*e.g.* ovalbumin). This is a consequence of the fact that a dendrimer, because of the molecular structure (tree shaped) generally has a lower molecular density, *i.e.* less compact compared to a protein. The higher molecular density of a protein is due to the ability to tightly fold the linear polypeptide chain into a three-dimensional structure by extensive intramolecular ion-pairing, hydrogen and hydrophobic bonding and disulfide cross-binding.[46] However, in comparison with conventional linear polymers, the dendrimers are generally more compact molecules taking up a smaller hydrodynamic volume.[47] X-ray analysis on supramolecular dendrimer aggregates has revealed that the molecular shape of the dendrimer upon increasing generation becomes increasingly globular (*i.e.* more spherical in contrast to linear shaped), in order to spread out the larger molecular structure with a minimal repulsion between the segments.[48]

Table 1.1 *Physicochemical properties of dendrimers in comparison to various biological entities*

Type of molecule	*Molecular weight*	*pI/surface charge*	*Diameter*	*Number and type of surface functional groups*[a]
G3-PAMAM (Starburst[†])	2411	/+	2.2 nm	12 primary amines
G6-PAMAM[‡]	28.788	11/+	6.5 nm	128 primary amines
G6-PAMAM-OH	28.913	9/0	—	128 hydroxyls
Medium sized protein (ovalbumin)	43.000	5/+ and –	5 nm	20 primary amines 10 phenol groups 4 thiols, 7 imidazoles
Large protein (Keyhole Limpet Hemocyanin)	~5.000.000	/+ and –	—	Approximately 2000 primary amines, 700 thiols, 1900 phenols
Virus	~40.000.000	—	50–200 nm	—
Prokaryotic bacteria	—	Mainly negative	1–2 μm (30 nm cell membrane and cell wall)	—
Eukaryotic cell	—	Mainly negative	20 μm (9 nm cell membrane)	—

[a]Protein functional groups not necessarily surface localised, [†]core group is trifunctional, branches are made up of ammonia and ethylenediamine building blocks; Starburst is a Trademark of Dendritech Inc., Midland, MI, US, [‡]core group is tetrafunctionalised, branches are made up of methyl acrylate and ethylenediamine building blocks.

The use of dendrimers as protein mimics has encouraged scientist to carry out studies to investigate the physicochemical properties of dendrimers in comparison to proteins. Being nano sized structures, dendrimers may respond to stimuli from the surroundings and can, like proteins, adapt a tight-packed conformation ("native") or an extended ("denaturated") conformation, depending on solvent, pH, ionic strength and temperature. However, there are some major differences in the molecular structures of dendrimers in comparison to proteins, resulting in a different physicochemical response of a dendrimer compared to a protein. The dendrimer architecture incorporates a high degree of conjunction consisting of a network of covalent bonds, which results in a somewhat less flexible structure than found in proteins.

Numerous of studies have been carried out to investigate the physicochemical properties of dendrimers applying computer simulations and chemical analytical techniques. And in order to optimise the computer models to give a realistic picture, a large amount of comparative studies have been carried out between predictions-based theoretical calculations and experimental results by chemical analysis.[49,50]

Dendrimers and the effect of molecular growth: The conformational behaviour of a dendrimer upon growing to higher generations are determined by (1) the molecular dimensions of the monomers–short monomers induce rapid proliferation of chains within a small space (2) the flexibility of the dendrons and (3) the ability of the end-groups to interact with each other, *e.g.* by hydrogen bonding creating a dense outer shell.

An initial attempt to predict the intramolecular behaviour of a dendrimer upon increasing the generation number using molecular simulations was reported by the French scientists De Gennes and Hervet,[51] who already in 1983 presented a modification of the "Edwards self-consistent field" theory to describe the conformational characteristics upon growth of a PAMAM (Starburst) dendrimer. Their analyses concluded that upon growth, the periphery (outer shell) of the dendrimer becomes increasingly crowded whereas the molecular density of the core region remains low throughout the molecular growth. As no back-folding (dendrons folding into the interior of the dendrimer) is taken into account, the increasing molecular crowding in the outer shell will give a limitation on the generation number that a starburst dendrimer can grow to.

One major problem in applying this model for dendrimers having, *e.g.* amine surface groups is that it does not take into account that the dendrons in these compounds have a relatively high mobility because of the lack of binding interactions between both the dendrimer arms and the functionalities at the surface. This larger mobility enables the dendrons to fold inwards towards the dendrimer interior as a consequence of entropy, disfavouring the more ordered De Gennes dense shell packing conformation.[49] Thus, the structural behaviour of the dendrimer upon growing to higher generations is determined by the ability of the surface functionalities to form a network with each other via, *e.g.* hydrogen bonding or ion pairing thereby consolidating a dense outer shell. For this reason, the "De Gennes model" has generally been opposed as a suitable model to describe unmodified flexible dendrimers as, *e.g.* amino-terminated PPI and PAMAM dendrimers.[50] However, in cases where the dendrimer contain surface groups capable of hydrogen bonding a dendrimeric motif with a very dense periphery (outer shell) and a hollow core may be obtained. An example of "dense-shell behaviour" has been investigated by Meijers group[52] who modified the surface amino groups of high-generation PPI dendrimers with Boc-phenyl alanine. Boc-phenyl alanine formed numerous of hydrogen bonds between the outer shell amides achieved by the amidation of the dendrimer. In case of the G5-PPI dendrimer, an outer shell was obtained with such a high molecular density that small molecules, *e.g.* Rose Bengal and *para*-nitrobenzoic acid could be entrapped inside the dendrimer without leakage to the surrounding solvent. This dense shell dendrimer was named "the dendritic box", and was besides being seminal in understanding fundamental structural chemistry of dendrimers, the first experimental report pointing towards using dendrimers as molecular containers, for *e.g.* drug delivery (see Chapter 3). Also, later studies of PPI dendrimers modified with amino acids capable of forming hydrogen bonds did show a good correlation with De Gennes "dense shell packing model" when increasing the generation number for these systems.[53] In this and similar cases the dense shell model of De Gennes and Hervet is followed, because the hydrogen bonding between the end-groups disfavour back-folding, which would otherwise lead to a higher molecular density in the interior of the dendrimer (Figure 1.13).

In order to give a more realistic picture on the molecular density in dendrimers having a more flexible structure Lescanet and Muthukumar[54] used "kinetic growth" simulations to predict the molecular conformation of the Starburst molecules. Using this approach they found that extensive back-folding may be found at the late stages

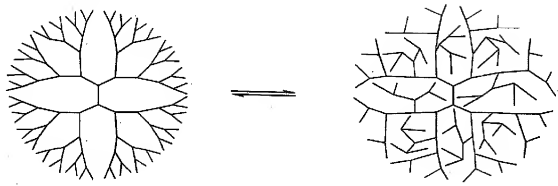


Figure 1.13 *Schematic depiction of the consequence of back-folding resulting in an increased molecular density in the interior of a dendrimer*

of dendritic growth. These predictions were confirmed by experimental observations performed on unmodified Starburst PAMAM amino-terminated dendrimers (G0–G7) using ^{2}H and ^{13}C NMR. By using the NMR correlation- and spin-lattice relaxation times, the mobility of the dendritic segments (dendrons) upon increasing generation could be measured. The carbon NMR experiments revealed no supression of mobility of the dendritic chain ends (termini), thus a low mobility of the chain ends is a condition for dense packing of functional groups on the dendrimer surface (*e.g.* De Gennes). In addition, increased average correlation times (τ) for the interior segments, indicated an increasing molecular density in the interior as a result of back-folding.[55] ^{2}H-NMR relaxation experiments, to study chain mobility, indicated a less restricted (faster) segmental motion of the chain ends (opposing the model of De Gennes) in comparison to the chains of the interior of the dendrimer.[56] These findings were in accordance with the molecular simulations reported by Lescanet and Muthukumar, approving this model to describe these types of dendrimers. Also, calculations based on molecular dynamics indicate that flexible dendrimers of all generations exhibit a dense core region and a less dense plateau region close to the periphery of the molecule, *i.e.* low generation dendrimers have conformations with low degree of back-folding ("density overlap") compared to higher generations. Upon reaching higher generations, the amount of back-folding increases upto the G8 dendrimers, where the molecular density is nearly uniform over the entire dendrimer.[57]

Comparative studies have been carried out to determine the shape and evaluate the change in steric interactions in amino-terminated PAMAM dendrimers compared to carbosilane dendrimers upon increasing generation. The steric repulsion is determined by the "scaled steric energy parameter". Carbosilane dendrimers are more spherical in shape compared to PAMAM with the smaller generation dendrimers being less spherical than the higher generation dendrimers. As carbosilane dendrimers

are more spherical, the higher generation dendrimers are capable of having an increased number of terminal groups on the molecular surface without increase of molecular density in the outer shell region. This may be due to silicon, being a third period element with a more flexible bond geometry. For PAMAM dendrimers, the steric repulsion becomes almost constant with G>4, whereas for carbosilane dendrimers the steric repulsion decreases upon increasing generation number.[58]

Dendrimers and the effect of pH: Amino-terminated PPI and PAMAM dendrimers have basic surface groups as well as a basic interior. For these types of dendrimers with interiors containing tertiary amines, the low pH region generally leads to extended conformations due to electrostatic repulsion between the positively charged ammonium groups.

Applying molecular dynamics to predict the structural behaviour of PAMAM dendrimers as a function of pH show that the dendrimer has an extended conformation, based on a highly ordered structure at low pH (pH≤ 4). At this pH, the interior is getting increasingly "hollow" as the generation number increases as a result of repulsion between the positively charged amines both at the dendrimer surface and the tertiary amines in the interior.

At neutral pH, back-folding occurs which may be a consequence of hydrogen bonding between the uncharged tertiary amines in the interior and the positively charged surface amines. At higher pH (pH≥ 10) the dendrimer contract as the charge of the molecule becomes neutral, aquiring a more spherical (globular) structure based on a loose compact network, where the repulsive forces between the dendrimer arms and between the surface groups reaches a minimum.[59] At this pH, the conformation has a higher degree of back-folding as a consequence of the weak "inter-dendron" repulsive forces (Figure 1.14).

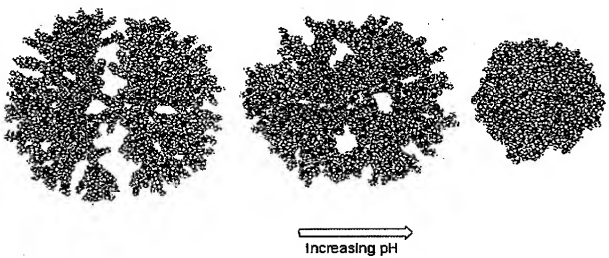


Figure 1.14 *Three-dimensional structure of a G6-PAMAM dendrimer, under different pH. Calculations is based on molecular dynamics*
(Reprinted from *Macromolecules*, 2002, **35**, 4510, with permission. ©2002, American Chemical Society)

Calculations as well as experimental data generally conclude that dendrimers (G5–G7) are conformationally more affected by change in pH and ionic strength in comparison to higher generation dendrimers (*e.g.* G8). The reason for this may be found in the somewhat more restricted motion of the outer shell chain segments in the higher generation dendrimers, leading to a more globular-shaped molecule despite different conditions in the surroundings.[60] As a curiosum, recent investigations show that amino-terminated PAMAM and PPI dendrimers in addition to their pH dependent conformational changes also fluoresce at low pH.[61]

When looking at the pH-dependent conformational changes of PPI dendrimers having acidic (carboxylic acid) end-groups, the picture is somewhat different compared to what is observed for their amino-terminated counterparts (Figure 1.15). Small angle neutron scattering (SANS) and NMR measurements of self-diffusion coefficients at different pH values show that at pH 2 the dendrimer core has the most extended conformation due to the electrostatic repulsion between the positively charged protonated tertiary amines, leading to a large radius of the core, whereas the dendrimer reaches its minimum radius at pH 6, where the amount of positively charged amines equals the amount of negatively charged carboxylic groups (isoelectric point) resulting in a "dense core" conformation more subjective to back-folding. Thus, at pH 6 some degree of back-folding occurs as a result of attractive Coulomb interactions between the negatively charged surface carboxy-groups and the positively charged tertiary amines in the inner shells of the dendrimer.[62] This shows that back-folding is not only a result of weak forces leading to a uniform molecular density of the dendrimer (entropy), but may also be mediated by attractive forces (enthalpy) between inner parts of the dendrons and surface groups. In the carboxy-PPI dendrimers a back-folded conformation minimise the repulsion between the negatively charged surface groups and between the positively charged inner shell amines leading to a lower repulsive energy of the system. At pH 11 the electrostatic repulsion between the negative charged forces the surface groups apart to give a more extended conformation with a highly expanded surface area (Figure 1.15).

Dendrimers and the effect of solvent: The ability of the solvent to solvate the dendrimer structure is a very important parameter when investigating the conformational state of a dendrimer. Molecular dynamics has been applied to study the variation of

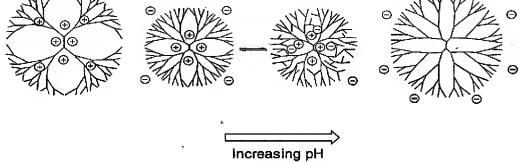


Figure 1.15 *Two-dimensional depiction of conformational changes upon different pH of a carboxy-terminated PPI-dendrimer*

dendrimer conformation as a function of dendrimer generation in different solvents.[57] Dendrimers of all generations generally all experience a larger extend of back-folding with decreasing solvent quality, *i.e.* decreasing solvation. However, being more flexible, the low generation dendrimers show the highest tendency towards back-folding as a result of poor solvation compared to the higher generation dendrimers.

NMR studies performed on PPI dendrimers conclude that an apolar solvent like benzene, poorly solvates the dendrons favouring intramolecular interactions between the dendrimer segments and back-folding. However, a weakly acidic solvent like chloroform can act as a hydrogen donor for the interior amines in a basic dendrimer like PPI, leading to an extended conformation of the dendrimer because of extensive hydrogen bonding between the solvent and the dendrimer amines.[63] Both experimental as well as theoretical studies on amino-terminated PPI and PAMAM dendrimers (polar dendrimers) show the tendency that apolar aprotic ("poor") solvents induce higher molecular densities in the core region as a result of back-folding, whereas polar ("good") solvents solvate the dendrimer arms and induce a higher molecular density on the dendrimer surface.

Interestingly, dendrimers having polar surface groups to some extent resemble proteins in their conformational behaviour when subjecting these structures to more apolar conditions, in the sense that back-folding of the polar surface groups may expose the more hydrophobic dendrimer parts to the surroundings leading to a decreased surface polarity of the back-folded dendrimer. A similar behaviour has been observed in the adsorption of proteins onto hydrophobic surfaces, giving a highly denaturated (unfolded) state of the protein exposing its interior hydrophobic regions to interact with the surface (Figure 1.16).[64]

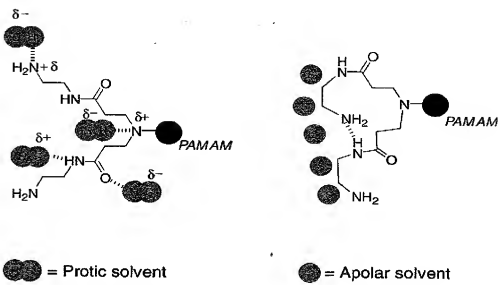


Figure 1.16 *Proposed scheme for solvation of a dendrimer under different solvent conditions. Left: Solvation of a polar dendrimer in a protic solvent ("good"), solvent leading to extended conformation exposing a polar surface. Right: Polar dendrimer in an apolar aprotic solvent ("poor"), solvent leading to exposure of an apolar surface consisting of alkyl chains by back-folding*

In dendrimers with an interior structure based on chiral mixed pyridine-dicarboxyanilide structures capable of hydrogen bonding, CD measurements showed that the dendrons were more temperature sensitive to unfolding processes in a polar solvent like acetonitrile compared to apolar solvents.[65] This may be explained from their more open and flexible structure, more easily accessible to solvation and H-bond disruption by polar solvents. The higher generation (G3) dendrons formed a more stable intramolecular network less prone to be "denaturated" by the solvent, resulting in higher denaturation temperatures for these dendrons.

When taking a look at dendrimers with less polar interior structures, *e.g.* dendrimers based on Fréchet type dendrons, the behaviour in various solvents is, as would be expected, significantly different from the more polar dendrimer constructs. For these, rather apolar π-reactive dendrimers, toluene proved to be a "good" solvent because of its ability to solvate the benzene containing Frechet dendrons by π-interactions. In toluene, the hydrodynamical volume was increased from G1 to G4 with strongest effect observed for the lower generations.[66] The increased solvation of the lower generations compared to higher generations may be a consequence of the more open structure of the low generation dendrimers allowing solvent molecules to penetrate into the interior of the dendrimer. A more polar solvent like acetonitrile, with a poor capability to solvate the dendrons, leads to a decrease in hydrodynamical volume indicative of increased intramolecular π–π interactions. The decrease in hydrodynamical volume was most pronounced for the G4 dendrimers.

Dendrimers and the effect of salt: Molecular simulations generally conclude that high ionic strength (high concentration of salts) has a strong effect on charged PPI dendrimers and favours a contracted conformation of dendrimers, with a high degree of back-folding somewhat similar to what is observed upon increasing pH or poor solvation.[67,68] At low salt conditions, the repulsive forces between the charged dendrimer segments results in an extended conformation in order to minimise charge repulsion in the structure (Figure 1.17).

Dendrimers and the effect of concentration: In dendrimers with flexible structures the conformation is not only affected by small molecules like solvents, salts or protons, but may also be sensitive to larger objects, such as other dendrimers or surfaces which can

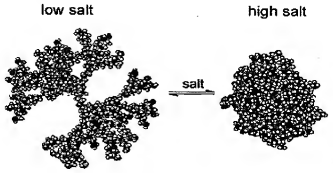


Figure 1.17 *Showing the three-dimensional conformational change of a PPI dendrimer upon increasing ionic strength*
(Reprinted from *Chemical Reviews*, 1999, **99**, 1665–1688, with permission. ©1999, American Chemical Society)

have a great affect on the molecular density and conformation of the dendrimer. Small angle X-ray scattering (SAXS) experiments performed on PPI dendrimers (G4, G5) in a polar solvent like methanol show that the molecular conformation of dendrimers upon increasing concentration becomes increasingly contracted. This molecular contraction may minimise the repulsive forces between the dendrimer molecules and increase the ability of the dendrimers to exhibit a more tight intermolecular packing.[69]

1.6 Summary

Dendrimers pose an exciting possibility for chemists to create macromolecular structures with a specifically tailored function or several functions. Dendrimers, like macromolecules found in biology, respond to the surrounding chemical environment showing altered conformational behaviour upon changes in, *e.g.* pH, solvent polarity and ionic strength.

When going from smaller dendritic structures to more globular macromolecular structures, compartments arise and the core region becomes increasingly shielded off from the surroundings by the dendritic wedges and an increasingly dense surface. The built-up dendrimer may be tailored to create a densely packed "De Gennes shell", *e.g.* by the introduction of hydrogen bonding surface groups or a more loose, flexible structure can be obtained by diminishing the attractive forces between the surface functionalities. In flexible dendrimer structures, back-folding may occur as a consequence of weak forces between the surface functionalities or dendrons leading to a more disordered conformation favoured by entropy, where the molecular density is spread out over the entire molecular area. However, back-folding may also be a result of attractive forces (ion-pairing, hydrogen bonding, π-interactions, *etc.*) between functional groups at the inner part of the dendrons and the surface functional groups. In these cases, back-folding is to a large extend driven by enthalpy. However, in both cases, the back-folded state may lead to a more low-energy state of the dendrimer. In addition, the degree of back-folding is to a large extend determined by the surroundings (solvent polarity, ionic strength), thereby constituting a delicate balance between intramolecular forces and forces applied by the surroundings.

The microenvironment in the core may be used to carry low-molecular substances, *e.g.* drugs, or may be useful to create altered properties of core-chromophores or fluorophores, *etc.* Furthermore, the dendrimers expose a multivalent surface, which as elsewhere in biology, is a promising motif to enhance a given functionality. In the next section the multivalency will be treated in more detail, how does the multivalency of the surface functionalities affect a given surface function in biological systems and how does these highly synthetic macromolecules interact with biological systems like cells, proteins and biological membranes *in vitro* and *in vivo*?

References

1. W.F. Ganong, *Review of Medical Physiology*, 15th edn, Prentice-Hall, New York, 1991.
2. K. Autumn, Y.A. Liang, S.T. Hsieh, W. Chan, T.W. Kenny, R. Fearing and R.J. Full, *Nature*, 2000, **405**, 681–685.

3. E. Buhleier, W. Wehner and F. Vögtle, *Synthesis*, 1978, 155–158.
4. D.A. Tomalia, J.R. Dewald, M.R. Hall, S.J. Martin and P.B. Smith, *Preprints 1st SPSJ Polym. Conf., Soc. Polym. Sci. Jpn., Kyoto*, 1984, 65.
5. D.A. Tomalia, H. Baker, J. Dewald, M. Hall, G. Kallos, S. Martin, J. Roeck, J. Ryder and P. Smith, *Polym. J.*, 1985, **17**, 117–132.
6. C. Wörner and R. Mühlhaupt, *Angew. Chem.*, 1993, **105**, 1367.
7. E.M.M. De Brander van den Berg and E.W. Meijer, *Angew. Chem.*, 1993, **105**, 1370.
8. J.P. Tam, *Proc. Natl. Acad. Sci. USA*, 1988, **85**, 5409.
9. F. Zeng and S.C. Zimmernan, *Chem. Rev.*, 1997, **97**, 1681.
10. A.W. Bosman, H.M. Janssen and E.W. Meijer, *Chem. Rev.*, 1999, **99**, 1665.
11. G.M. Dykes, *J. Chem. Technol. Biotechnol.*, 2001, **76**, 903.
12. K. Sadler and J.P. Tam, *Rev. Mol. Biotechnol.*, 2002, **90**, 195.
13. M.J. Cloninger, *Curr. Opin. Chem. Biol.*, 2002, **6**, 742.
14. U. Boas and P.M.H. Heegaard, *Chem. Soc. Rev.*, 2004, **33**, 43.
15. T. Emrick and J.M.J. Fréchet, *Curr. Opin. Colloid. Interface Sci.*, 1999, **4**, 15.
16. D.A. Tomalia, D.M. Hedstrand and M.S. Ferritto, *Macromolecules*, 1991, **24**, 1435.
17. C. Hawker and J.M.J. Fréchet, *J. Chem. Soc. Chem. Commun.*, 1990, 1010.
18. C.J. Hawker and J.M.J. Fréchet, *J. Am. Chem. Soc.*, 1990, **112**, 7638.
19. C.J. Hawker, K.L. Wooley and J.M.J. Fréchet, *J. Chem. Soc. Perkin. Trans. 1*, 1993, 1287.
20. J.P. Tam, Synthesis of peptides and peptidomimetics, Houben-Weyl Methods of organic chemistry, in *Peptide Dendrimers and Protein Mimetics*, M. Goodman (ed), Thieme, Stuttgart, 2000.
21. Commercially available by dendritech, www.dendritech.com.
22. G.R. Newkome, C.N. Moorefield, G.R. Baker, M.J. Saunders and S.H. Grossman, *Angew. Chem. Int. Ed. Eng.*, 1991, **30**, 1178.
23. J.-P. Majoral and A.-M. Caminade, *Chem. Rev.*, 1999, **99**, 845.
24. W.B. Turnbull and J.F. Stoddart, *Rev. Mol. Biotechnol.*, 2002, **90**, 231.
25. R.H.E. Hudson and M.J. Damha, *J. Am. Chem. Soc.*, 1993, **115**, 2119.
26. R.H.E. Hudson, S. Robidoux and M.J. Damha, *Tetrahedron Lett.*, 1998, **39**, 1299.
27. T.W. Nilsen, J. Grazel and W. Prensky, *J. Theor. Biol.*, 1997, **187**, 273.
28. G.R. Newkome, E. He and C. Moorefield, *Chem. Rev.*, 1999, **99**, 1689.
29. M.K. Lothian-Tomalia, D.M. Hedstrand and D.A. Tomalia, *Tetrahedron*, 1997, **53**, 15495.
30. J.R. Morgan and M.J Cloninger, *Curr. Opin. Drug Discov. Develop.*, 2002, **5**, 966.
31. F. Vögtle, H. Fakhrnabavi, O. Lukin, S. Müller, J. Friedhofen and C.A. Schally, *Eur. J. Org. Chem.*, 2004, 4717.
32. E.T. Kaiser, H. Mihara, G.A. Laforet, J.W. Kelly, L. Walters, M.A. Findeis and T. Sasaki, *Science*, 1989, **243**, 187.
33. K.L. Wooley, C.J. Hawker and J.M.J. Fréchet, *J. Am. Chem. Soc.*, 1993, **115**, 11496.
34. S.C. Zimmerman, F. Zeng, D.E.C. Reichert and S.V. Kolotuchin, *Science*, 1996, **271**, 1095.

35. A.W. Freeman, R. Vreekamp and J.M.J. Fréchet, *Abstr. Pap. Am. Chem. Soc.*, 1997, **214**, 128-PMSE.
36. P.S. Corbin, L.J. Lawless, Z. Li, Y. Ma, M.J. Witmer and S.C. Zimmerman, *Proc. Natl. Acad. Sci. USA*, 2002, **99**, 5099.
37. C.R. DeMattei, B. Huang and D.A. Tomalia, *Nano Lett.*, 2004, **4**, 771.
38. Y. Choi, T. Thomas, A. Kotlyar, M.T. Islam and J.R. Baker, *Chem. Biol.*, 2005, **12**, 35.
39. M. Kawa and J.M.J. Fréchet, *Chem. Mater.*, 1998, **10**, 286.
40. V.V. Narayanan and E.C. Wiener, *Macromolecules*, 2000, **33**, 3944.
41. V. Percec, C.-H. Ahn, G. Ungar, D.J.P. Yeardley, M. Möller and S.S. Sheiko, *Nature*, 1998, **391**, 161.
42. M. Numata, A. Ikeda and S. Shinkai, *Chem. Lett.*, 2000, 370.
43. T.E. Creighton, *Proteins, Structures and Molecular Properties*, 2nd edn, W.H. Freeman, New York.
44. C. Kojima, K. Kono, K. Maruyama and T. Takagishi, *Bioconjugate Chem.*, 2000, **11**, 910.
45. D. Farin and D. Avnir, *Angew. Chem. Int. Ed. Engl.*, 1991, **30**, 1379.
46. A. Nourse, D.B. Millar and A.P. Minton, *Biopolymers*, 2000, **53**, 316.
47. G.M. Pavlov, E.V. Korneeva and E.W. Meijer, *Colloid Polym. Sci.*, 2002, **280**, 416.
48. V. Percec, W.-D. Cho, P.E. Mosier, G. Ungar and D.J.P. Yeardley, *J. Am. Chem. Soc.*, 1998, **120**, 11061.
49. M. Ballauf, *Topics Curr. Chem. Dendrimers III: Design, dimension, function.* 2001, **212**, 177.
50. M. Ballauf and C.L. Likos, *Angew. Chem. Int. Ed. Engl.*, 2004, **43**, 2998.
51. P.G. DeGennes and H. Hervet, *J. Phys. Lett. Paris*, 1983, **44**, L351.
52. J.F.G.A. Jansen, E.M.M. De Brabander van den Berg and E.W. Meijer, *Science*, 1994, **266**, 1226.
53. A.W. Bosman, M.J. Bruining, H. Kooijman, A.L. Spek, R.A.J. Janssen and E.W. Meijer, *J. Am. Chem. Soc.*, 1998, **120**, 8547.
54. R.L. Lescanet and M. Muthukumar, *Macromolecules*, 1990, **23**, 2280.
55. A.D. Meltzer, D. Tirrel, A.A. Jones, P.T. Inglefield, D.M. Hedstrand and D.A. Tomalia, *Macromolecules*, 1992, **25**, 4541.
56. A.D. Meltzer, D. Tirrel, A.A. Jones and P.T. Inglefield, *Macromolecules*, 1992, **25**, 4549.
57. M. Murat and G.S. Grest, *Macromolecules*, 1996, **29**, 1278.
58. M. Elshakre, A.S. Atallah, S. Santos and S. Grigoras, *Comput. Theor. Polym. Sci.*, 2000, **10**, 21.
59. I. Lee, B.D. Athey, A.W. Wetzel, W. Meixner and J.R. Baker, *Macromolecules*, 2002, **35**, 4510.
60. T. Terao and T. Nakayama, *Macromolecules*, 2004, **37**, 4686.
61. D.J. Wang and T. Imae, *J. Am. Chem. Soc.*, 2004, **126**, 13204.
62. I.B. Rietveld, W.G. Bouwman, M.W.P.L. Baars and R.K. Heenan, *Macromolecules*, 2001, **34**, 8380.
63. M. Chai, Y. Niu, W.J. Youngs and P.L. Rinaldi, *J. Am. Chem. Soc.*, 2001, **123**, 4670.

64. J.E. Butler, L. Ni, R. Nessler, K.S. Joshi, M. Suter, B. Rosenberg, J. Chang, W.R. Brown and L.A. Cantaro, *J. Immunol. Methods*, 1992, **150**, 77.
65. J. Recker, D.J. Tomcik and J.R. Parquette, *J. Am. Chem. Soc.*, 2000, **122**, 10298.
66. S. De Backer, Y. Prinzie, W. Verheijen, M. Smet, K. Desmedt, W. Dehaen and F.C. De Schryver, *J. Phys. Chem. A*, 1998, **102**, 5451.
67. P. Welch and M. Muthukumar, *Macromolecules*, 1998, **31**, 5892.
68. A. Ramzi, R. Scherrenberg, J. Joosten, P. Lemstra and K. Mortensen, *Macromolecules*, 2002, **35**, 827.
69. A. Topp, B.J. Bauer, T.J. Prosa, R. Scherrenberg and E.J. Amis, *Macromolecules*, 1999, **32**, 8923.